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The study aimed to identify genetic lesions associated with secondary acute myeloid leukemia (sAML) in comparison with AML arising de novo (dnAML) and assess their impact on patients' overall survival (OS). High-resolution genotyping and loss of heterozygosity mapping was performed on DNA samples from 86 sAML and 117 dnAML patients, using Affymetrix Genome-Wide Human SNP 6.0 arrays. Genes *TP53*, *RUNX1*, *CBL*, *IDH1/2*, *NRAS*, *NPM1*, and *FLT3* were analyzed for mutations in all patients. We identified 36 recurrent cytogenetic aberrations (more than five events). Mutations in *TP53*, 9pUPD, and del7q (targeting *CUX1* locus) were significantly associated with sAML, while *NPM1* and *FLT3* mutations associated with dnAML. Patients with sAML carrying *TP53* mutations demonstrated lower 1-year OS rate than those with wild-type *TP53* (14.3% ± 9.4% vs. 35.4% ± 7.2%; $P = 0.002$), while complex karyotype, del7q (*CUX1*) and del7p (*IKZF1*) showed no significant effect on OS. Multivariate analysis confirmed that mutant *TP53* was the only independent adverse prognostic factor for OS in sAML (hazard ratio 2.67; 95% CI: 1.33–5.37; $P = 0.006$). Patients with dnAML and complex karyotype carried sAML-associated defects (*TP53* defects in 54.5%, deletions targeting *FOXP1* and *ETV6* loci in 45.4% of the cases). We identified several co-occurring lesions associated with either sAML or dnAML diagnosis. Our data suggest that distinct genetic lesions drive leukemogenesis in sAML. High karyotype complexity of sAML patients does not influence OS. Somatic mutations in *TP53* are the only independent adverse prognostic factor in sAML. Patients with dnAML and complex karyotype show genetic features associated with sAML and myeloproliferative neoplasms. Am. J. Hematol. 87:1010–1016, 2012. © 2012 Wiley Periodicals, Inc.

Introduction

The continuous production of terminally differentiated blood cells in the hematopoietic system is a tightly regulated process involving self-renewal, proliferation, and differentiation of stem and progenitor cells. Disruption of this process by acquired genetic lesions may cause the dominance of stem cell clones with variable output of myeloid cells. As a consequence, the production of terminally differentiated cells may be excessive resulting in myeloproliferative neoplasms (MPN) or deficient accompanied with dysplasia, with or without the presence of blasts, resulting in myelodysplastic syndromes (MDS).

The classic *BCR-ABL*-negative MPNs include three disease entities—polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) [1]. In 95% of PV and approximately half of ET and PMF cases the initiation of the clinical phenotype is hallmarked by somatic mutations in the *JAK2* gene [2–5], often amplified to homozygosity by uniparental disomy (UPD) of chromosome 9p [6]. MDS are a heterogeneous group of disorders and a

Additional Supporting Information may be found in the online version of this article.

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number of genetic lesions have been implicated in their pathogenesis, including del5q [7], as well as mutations in the RNA splicing pathway [8], *TET2* [9], *EZH2* [10], and other genes. MPN and MDS are chronic disorders with an elevated risk of disease progression to secondary acute myeloid leukemia (sAML). The rate of leukemic transformation in *BCR-ABL*-negative MPN patients is 7% [11], while MDS patients transform in 30% of the cases [12]. Disease progression to sAML is characterized by the presence of >20% of blasts in the bone marrow and sequential acquisition of genetic aberrations [13,14]. Although several studies on limited patient cohorts showed association of gene mutations and cytogenetic aberrations with leukemic transformation of MPN and MDS, the leukemogenesis process remains poorly understood. Mutations in *FLT3*, *NRAS* [15], *NPM1* [16], *RUNX1* [17], *DNMT3A* [18], *IDH1*, *IDH2* [19], *TET2* [20,21], and *TP53* [22] have been implicated in leukemic transformation, as well as several chromosomal aberrations, such as deletions of *IKZF1* [23], *JARID2*, *AEBP2* [24], and amplifications of *MDM4* [22].

Studies of clonal hierarchy showed that sAML can arise on the background of the chronic phase founder clone or alternatively as an independent event, resembling dnAML [25–27]. Unlike dnAML patients who often achieve complete remission after treatment, patients who transform to sAML have very poor prognosis and die within a few months following AML diagnosis [28], suggesting that leukemogenesis must differ substantially between sAML and dnAML.

Since the genetic basis of sAML is mainly unknown, the aim of our study was to delineate genetic profiles of sAML patients using high-resolution genome-wide single-nucleotide polymorphism (SNP) arrays, as well as direct sequencing of genes known to be involved in AML pathogenesis. Furthermore, we aimed to compare the frequency of recurrent genetic lesions in sAML and dnAML, and analyze their prognostic significance in order to define which genetic aberrations account for the poor prognosis of sAML patients. We used a bioinformatic approach to study co-occurrence of genetic aberrations and their association with patient diagnosis, in order to identify new genes involved in leukemogenesis and define potential new markers that would allow better genetic stratification of AML patients.

Methods

Patient samples. A total of 203 patients were included in this study, 117 diagnosed with dnAML and 86 with sAML. Patients diagnosed with therapy-related AML were not included in the study. Patients were diagnosed as sAML according to the 2008 WHO classification, and all samples were collected at the time of sAML diagnosis. Time to leukemic transformation was measured from the time of MPN/MDS diagnosis to the date of sAML diagnosis. Clinical data were available for 184 patients (110 dnAML and 74 sAML). Classical cytogenetic analysis was performed according to routine cytogenetic procedures, using GTG-banding technique. Peripheral blood samples from patients were collected from institutions in Italy, Austria, Czech Republic, and Serbia, following the local ethical regulations. Genomic DNA was isolated from granulocytes, bone marrow, or whole blood samples, following standard protocols.

Microarray analysis. All patients' DNA samples were processed and hybridized to Genome-Wide Human SNP 6.0 arrays (Affymetrix, Santa Clara, CA) according to the manufacturer's instructions. Raw data were analyzed using Genotyping Console Version 3.0.1 software (Affymetrix, Santa Clara, CA) for quality, identification of copy number alterations, and losses of heterozygosity. Detected chromosomal aberrations (gains, deletions, and UPDs) were annotated. The criteria for annotating UPD regions were terminal location on the chromosome and size of >1 Mb. UPDs found in patients with numerous interstitial runs of homozygosity (>10 Mb) were excluded from further analysis, as they infer parental consanguinity [29]. All aberrations mapping to known copy number variation loci according to the Database of Genomic Variants (DGV Version 5, human reference genome assembly hg18) were not annotated.

Mutational analysis. Exon sequencing of all coding exons of *RUNX1*, *TP53*, as well as exon 1 of *NRAS*, exons 8 and 9 of *CBL*, exons 4 of *IDH1* and *IDH2* was performed using BigDye Terminator Version 3.1 cycle-sequencing kit and the 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequencher Version 4.9 software (Gene Codes Corporation, Ann Arbor, MI) was used for sequence analysis. Screening of *FLT3* internal tandem duplications, *FLT3* mutations at position D835 and insertions in exon 12 of *NPM1* were performed as previously described [30,31]. All primer sequences are listed in Supporting Information Table I.

Statistical analysis. Patient characteristics, frequencies of mutations, and chromosomal aberrations were compared using the Fisher's exact test for categorical data and Mann-Whitney test for continuous data. OS was measured from the date of AML diagnosis to time of death from any cause. Patients were censored at the time of the last follow-up. In the sAML patient cohort 52 patients died during the follow-up, while 22 patients were censored. Survival curves were constructed using the Kaplan-Meier method. The comparison of OS curves was performed using the log-rank test and multivariate analysis of OS using the Cox regression model. All calculated *P* values are two-tailed. SPSS 20.0 (SPSS, Chicago, IL) statistical software package was used for statistical analyses. The negative interaction information analysis was performed as described in the Supporting Information methods.

Results

Patient characteristics

The study included a total of 203 patient samples, diagnosed with sAML ($N = 86$) or de novo AML ($N = 117$). The sAML patient cohort consisted of 48 patients with a previous diagnosis of MPN, 36 with MDS, and 2 with MPN/MDS overlap. The median duration of chronic phase before transformation was 5 years (range 0.2–22.8). Clinical characteristics of the patient cohort are summarized in Supporting Information Table II. Patients with sAML were treated according to different therapeutic protocols (Supporting Information Table III), however, this did not influence the outcome since most sAML patients did not respond to therapy. Nine sAML patients received allogeneic hematopoietic stem cell transplantation.

Frequencies of mutations in genes associated with AML differ between sAML and dnAML

To investigate whether the frequency of mutations in genes commonly affected in dnAML is the same in sAML we screened for mutations in *FLT3*, *NPM1*, *TP53*, *CBL*, *IDH1*, *IDH2*, *RUNX1*, and *NRAS* (Fig. 1A) in both patient groups. Within the sAML patient cohort we did not observe any significant difference in frequency of mutations with respect to the previous diagnosis (Supporting Information Fig. 2). At least one gene mutation was found in 56.41% of dnAML and 52.38% of sAML patients. In dnAML patients the most frequent were *NPM1* mutations, present in 24.79% (29 of 17) of cases, followed by *FLT3* mutations found in 23.93% (28 of 117) of patients. The frequency of these mutations was significantly lower in sAML patients, where *NPM1* was mutated in 4.94% and *FLT3* in 7.23% of cases ($P = 0.002$ and $P = 0.016$, respectively). In contrast, *TP53* mutations were found in 16.67% of sAML and only 4.27% of dnAML cases ($P = 0.046$). The frequencies of mutations in *RUNX1*, *IDH1*, *IDH2, CBL*, and *NRAS* were not significantly different in the two patient groups (Fig. 1A). *TP53* and *RUNX1* mutations mainly affected the DNA-binding domains of these proteins (Supporting Information Fig. 1). The list of all mutations in analyzed genes and their PolyPhen-2 prediction scores of functional effects are listed in Supporting Information Table IV. Furthermore, we observed that certain mutations co-occur, while others show mutual exclusivity (Fig. 1B–E). Co-occurrence of *NPM1* and *FLT3* mutations was observed in dnAML only, while *RUNX1* and *FLT3* mutations co-occurred in 4 dnAML and 2 sAML patients (Fig. 1B,C). In both patient groups, *IDH1* and *IDH2* mutations were mutually exclusive. Mutations in *TP53* were found to be mutually exclusive with *IDH1/2* in sAML (Fig. 1E).

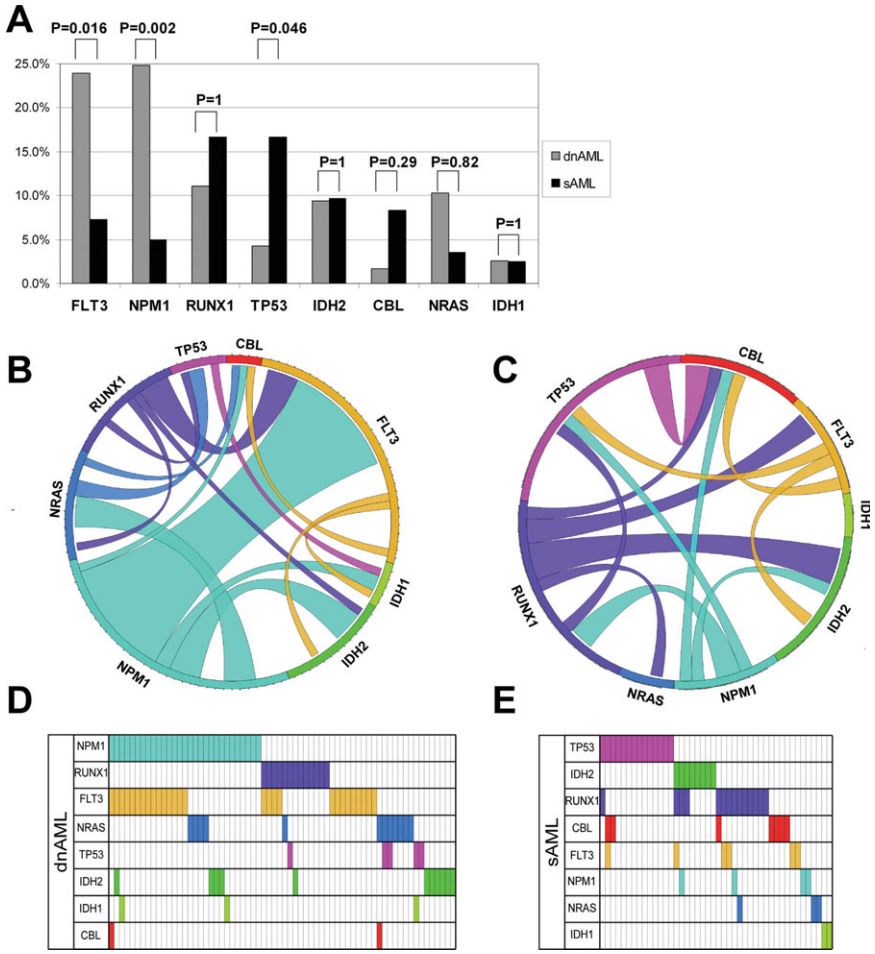

Figure 1. Gene mutation profiles in secondary (sAML) and de novo acute myeloid leukemia (dnAML). (A) Comparison of mutational frequencies in genes affected in myeloid malignancies shows significant bias of *FLT3* and *NPM1* mutations toward dnAML and *TP53* toward sAML. All *P* values have been corrected for multiple testing using the Bonferroni correction. The co-occurrence of mutations in dnAML (B) and sAML (C) is shown with Circos diagrams [32]. The length of the arc corresponds to the frequency of mutations in the individual gene, while the width of the ribbons connecting two arcs corresponds to the number of patients carrying both mutations. Certain mutations show mutual exclusivity in both dnAML (D) and sAML (E). Each vertical line represents one patient with at least one mutation.

Microarray-based karyotyping of sAML and dnAML

To get a deeper insight into the genetic complexity of sAML and dnAML, we performed high-resolution genome-wide analysis of DNA copy number abnormalities and losses of heterozygosity on 200 samples (114 dnAML and 86 sAML) using Affymetrix Genome-Wide SNP 6.0 arrays. Comparing with only 11.4% of dnAML cases, 44.2% of sAML patients presented with a complex karyotype ($P < 0.0001$), defined as more than or equal to three unrelated chromosomal aberrations not included in the WHO 2008 classification criteria [13]. In 17.4% (15 of 86) of the sAML cases we could not detect any chromosomal aberration, which was significantly lower comparing with 37.7% (43 of 114) of such dnAML patients ($P = 0.0017$). In the remaining samples we detected a total of 669 chromosomal aberrations, mainly deletions (59%, 395 of 669), but also gains (27.95%, 187 of 669) and UPDs (13%, 87 of 669) (Fig. 2). We found 36 recurrent chromosomal aberrations, present in more than five patients in our cohort (Table I). High number of deletions allowed the fine mapping of common deleted regions (CDR) to <1 Mb on specific chromosomal arms, pointing out the possible target genes, such as *CUX1*, *IKZF1*, *TET2*, *JARID2*, *SUZ12*, *RUNX1, TET1*, and *NF1*.

We compared the frequency of recurrent chromosomal aberrations in the two patient groups and found that 18 out of 36 recurrent cytogenetic aberrations show a bias toward sAML. However, after applying Bonferroni correction for multiple testing, only four chromosomal aberrations significantly associated with sAML (Table I). The strongest association was observed for 9pUPD, as a consequence of high prevalence of this aberration in chronic phase of MPN. The second strongest association was observed between sAML and del7q22.1 mapping to tumor suppressor *CUX1* locus. Interestingly, gains of chromosome 1q32.1, targeting the *MDM4* locus, were found exclusively in sAML patients ($N = 6$).

We also compared the frequency of recurrent chromosomal aberrations in sAML patients who developed AML following MPN or MDS chronic phase, and found that besides 9pUPD which showed association with previous MPN diagnosis, all other aberrations occurred in similar frequencies in both post-MPN and post-MDS AML (Supporting Information Table V).

All patients with 13qUPD ($N = 7$) and 17pUPD ($N = 4$) were carrying homozygous mutations in *FLT3* and *TP53*, respectively. Both *TP53* alleles were affected in 13 out of 19 patients with *TP53* mutations (Supporting Information Table VI). *CBL* mutations were amplified by 11qUPD in four cases, however, three sAML patients with 11qUPD did not carry mutations in *CBL*.

Association of recurrent genetic lesions

In order to analyze the association of recurrent genetic lesions, we performed Fisher's exact test on all pairs of an-

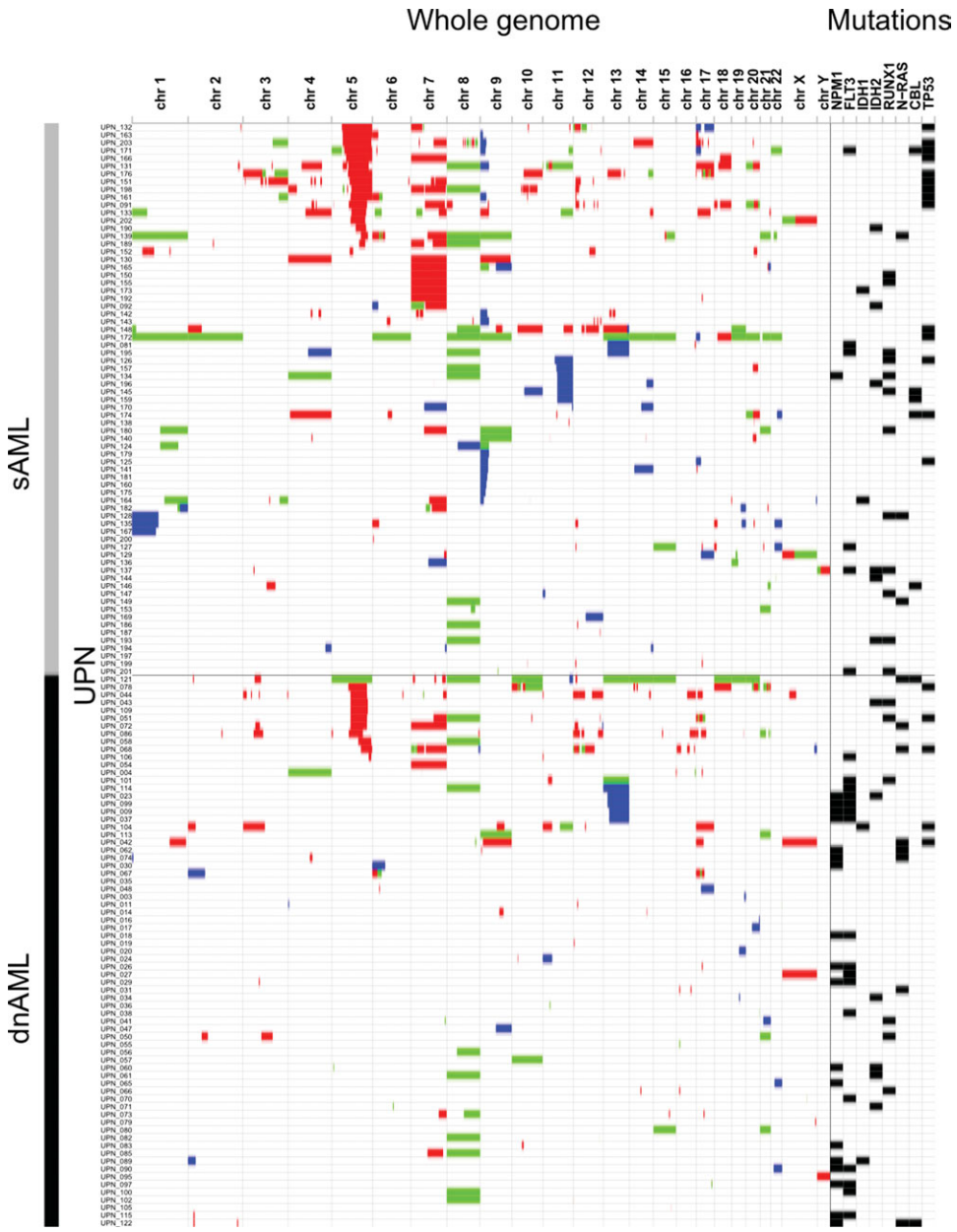

Figure 2. Whole-genome view of all chromosomal aberrations and gene mutations in 86 sAML and 117 dnAML patients. Each line represents the whole genome of one patient in which at least one genetic aberration was detected with Affymetrix Genome-Wide Human SNP 6.0 arrays. The size and physical position of each chromosomal aberration is represented with a colored bar. Deletions are represented in red, gains in green, and uniparental disomies in blue. Mutations in analyzed genes are shown as filled boxes. UPN, unique patient number.

alyzed gene mutations and recurrent cytogenetic aberrations (more than five events), provided they are located on different chromosomes. Supporting Information Table VII lists all significant aberration pairs ($N = 16$). Del5q was found to be associated with *TP53* mutations ($P = 0.0004$), del12p targeting *ETV6* locus ($P = 0.0022$), del17p ($P = 0.0159$), and del7q ($P = 0.0251$). We also found association of del18q with gain of 20q ($P = 0.0033$) and del4q targeting *TET2* locus with del20q ($P = 0.0153$).

The negative interaction information analysis was performed to detect the pairs of aberrations that have different patterns of association (co-occurrence or mutual exclusivity) depending on the disease subtype. The top 100 hits obtained from this analysis are listed in Supporting Information Table VIII. We observed that del5q frequently occurs together with 9pUPD and *TP53* mutations in sAML patients, and that *TP53* mutations and del10q targeting *TET1* gene locus are present at the same time in four sAML patients, while these aberrations never co-occur in dnAML.

Prognostic significance of recurrent genetic lesions

In order to define which genetic feature had the most adverse effect on the survival of patients with sAML, we analyzed the influence of mutations in *TP53*, del7q (*CUX1*), del7p (*IKZF1*), and presence of complex karyotype. These features were chosen because they were found to associate with sAML and are rare in both dnAML and chronic phase of MPN and MDS [31]. We focused our analysis on overall survival since most of the sAML patients did not respond to therapy or died early following sAML diagnosis. Patients with sAML carrying *TP53* mutations showed a significantly shorter median OS (1.8 months, 95% CI, 0.8–2.8), compared with patients with wild-type *TP53* (5.6 months, 95% CI, 1.5–9.7; $P = 0.002$) (Fig. 3A). Karyotype complexity did not show a significant impact on OS of sAML patients. The

TABLE I. Association of Recurrent (More Than Five Events) Chromosomal Aberrations with Patient Diagnosis

Chromosomal aberration	Start position	End position	Chromosomal band	% of sAML patients	% of dnAML patients	Total no. events	Target gene locus	*P*	*P* value after Bonferroni correction
9p UPD	1	9,000,000	9p24.3	17.4	0	15		<0.001	<0.01
7q LOSS	101,350,000	102,000,000	7q22.1	20.9	4.4	23	*CUX1*	<0.001	0.018
7q LOSS	65,180,000	65,830,000	7q11.21	13.9	1.75	14		0.001	0.043
7q LOSS	148,400,000	150,400,000	7q36.1	22.1	6.1	26		0.001	0.043
20q LOSS	35,000,000	35,100,000	20q11.23	8.1	0	7		0.002	0.086
4q LOSS	106,000,000	10,664,0000	4q24	10.5	0.9	10	*TET2*	0.005	0.191
20q LOSS	36,000,000	36,170,000	20q11.23	9.3	0.9	9		0.005	0.198
1q GAIN	201,000,000	204,496,000	1q32.1	7	0	6		0.006	0.205
9q GAIN	78,900,000	79,650,000	9q21	7	0	6		0.006	0.205
20q LOSS	33,500,000	34,000,000	20q11	7	0	6		0.006	0.205
7p LOSS	11,000,000	18,000,000	7p21	11.6	2.6	13		0.017	0.63
7p LOSS	50,080,000	50,600,000	7p12.2	13.9	4.4	17	*IKZF1*	0.021	0.752
7p LOSS	38,200,000	38,390,000	7p14.1	12.8	3.5	15		0.027	0.968
5q LOSS	130,900,000	139,400,000	5q31	18.6	7.9	25		0.030	1
5q LOSS	89,489,000	95,350,000	5q14->q15	16.3	6.1	21		0.034	1
Monosomy 7				8.1	1.7	9		0.040	1
9p GAIN	1	38,761,000	9p24	7	0.9	7		0.044	1
11q UPD	118,500,000	134,452,384	11q23.3->qter	7	0.9	7		0.044	1
6p LOSS	5,150,000	5,460,000	6p25.1	5.8	0.9	6		0.086	1
6p LOSS	14,130,000	15,800,000	6p23	5.8	0.9	6	*JARID2*	0.086	1
6p LOSS	19,955,000	20,820,000	6p22.3	5.8	0.9	6		0.086	1
18q LOSS	75,100,000	75,950,000	18q23	5.8	0.9	6		0.086	1
12p LOSS	11,800,000	12,900,000	12p13	10.5	4.4	14	*ETV6*	0.159	1
3p LOSS	71,600,000	73,000,000	3p13	1.2	5.3	7	*FOXP1*	0.242	1
Trisomy 8				12.8	8	18		0.341	1
20q GAIN	29,300,000	30,520,000	20q11.21	4.6	1.7	6		0.405	1
17q LOSS	26,000,000	26,600,000	17q11.2	9.3	6.1	15	*NF1*	0.427	1
21q GAIN	45,700,000	46,400,000	21q22.3	5.8	3.5	9		0.503	1
21q GAIN	36,300,000	40,250,000	21q22	5.8	4.4	10		0.748	1
17q LOSS	27,200,000	27,400,000	17q11.2	8.1	7	15	*SUZ12*	0.792	1
9q LOSS	92,300,000	93,850,000	9q22	2.3	3.5	6		1	1
13q UPD	106,100,000	114,142,980	13q33->qter	3.5	4.4	8		1	1
21q GAIN	13,280,000	27,000,000	21q11->q21	4.6	4.4	9		1	1
12q LOSS	92,900,000	93,400,000	12q22	3.5	2.6	6		1	1
17p LOSS	4,850,000	5,170,000	17p13.2	4.6	4.4	9		1	1
17p LOSS	7,080,000	8,080,000	17p13.1	4.6	5.3	10		1	1

UPD, uniparental disomy; sAML, secondary acute myeloid leukemia; dnAML, de novo acute myeloid leukemia.

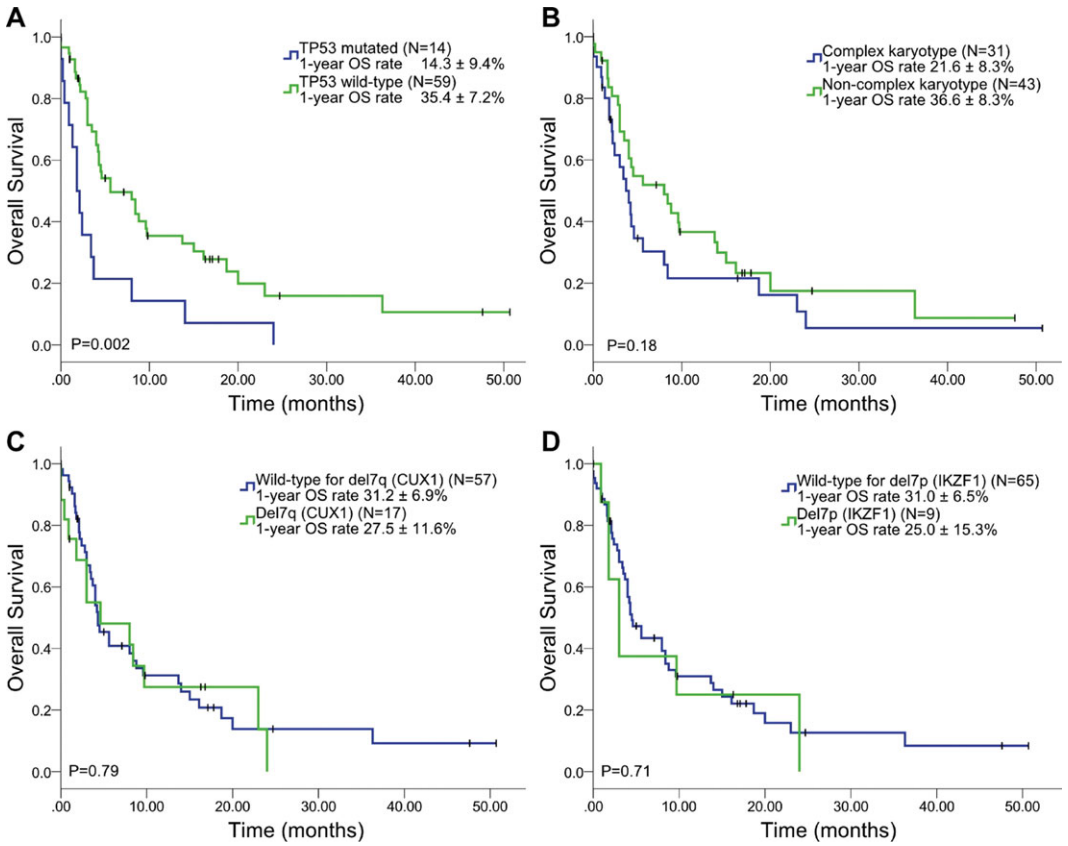

Figure 3. Impact of recurrent genetic lesions and karyotype complexity on overall survival (OS) of patients with secondary acute myeloid leukemia (sAML) represented by Kaplan-Meier curves. (A) OS in sAML patients with mutated (N = 14) or wild-type *TP53* (N = 59). (B) OS in sAML patients with complex (N = 31) or noncomplex (N = 43) karyotype. (C) OS of sAML patients according to presence (N = 17) or absence (N = 57) of del7q targeting *CUX1* locus. (D) OS of sAML patients according to presence (N = 9) or absence (N = 65) of del7p targeting *IKZF1* locus.

median survival of sAML patients with complex karyotype was 4 months (95% CI, 2.5–5.5), while the OS of patients with noncomplex karyotype was 8 months (95% CI, 3.0–13.0) (Fig. 3B). The effect of del7q (*CUX1*) and del7p (*IKZF1*) on OS of sAML patients was not significant (Fig. 3C,D). We included age, complex karyotype, mutations in *TP53*, del7p, and del7q in the model and performed multivariate analysis of OS of sAML patients. The multivariate analysis confirmed that mutated *TP53* was the only independent adverse prognostic factor for OS (hazard ratio 2.67; 95%CI, 1.33–5.37; $P = 0.006$) in sAML (Table II).

De novo AML patients with complex karyotype exhibit genetic features of secondary AML

There were 11 dnAML patients with complex karyotype in our patient cohort with available clinical data. The median OS of these patients was 3 months (95% CI, 2.0–4.0), and all patients died during the follow-up. To gain better understanding of poor OS of patients with complex karyotype within the dnAML group we looked at genetic profiles of each of these patients (Table III) and found that *TP53* was affected in 54.5% ($N = 6$ of 11) of these patients. *TP53* mutations were present in three patients, and additional three patients carried a deletion of *TP53* locus on chromosome 17p. *TP53* mutations were only present in this subtype of dnAML. We also found del7p (*IKZF1*) and del7q (*CUX1*) in four and three patients, respectively. In addition, these patients carried deletions mapping to the transcription factors *FOXP1* (45.4%, $N = 5$ of 11) and *ETV6* (45.4%, $N = 5$ of 11) (Table III). Taken together we observed a number of genetic features present in dnAML with complex karyotype typical for either MPN or sAML.

Discussion

The objective of this study was to examine to what extent the genetic basis of leukemogenesis in post-MPN and post-MDS AML differs from dnAML, by systematic analysis of genetic profiles of sAML and dnAML patients. We grouped the patients who developed sAML after MPN or MDS chronic phase, since we did not observe any genetic difference between the two groups in the leukemic stage. The comparison of mutational frequency in genes known to be affected in myeloid malignancies showed that *TP53* mutations significantly associated with sAML, whereas *NPM1* and *FLT3* were prominently involved in dnAML. *IDH1/2*, *NRAS*, *RUNX1*, and *CBL* mutations seem to be universally contributing to dnAML and sAML leukemogenesis. The mutual exclusivity of certain mutations such as *IDH1/2* with *CBL*, *TP53* and with *NRAS* confirms that distinct leukemogenic pathways are involved in sAML [33].

We extended the genetic marker analysis by karyotyping patients with high-resolution SNP arrays. The high number of chromosomal aberrations observed enabled us to fine map CDRs to several putative tumor suppressor gene loci (Table I). Compared with dnAML, we observed higher karyotype complexity in sAML and identified a number of cytogenetic lesions differentially distributed in both AML groups. Previous attempts to identify karyotype differences between sAML and dnAML were limited with the use of classical cytogenetic methods, although the association of del7q with sAML was previously reported [34]. Association of del7q was recently confirmed by SNP array karyotyping and the 7q CDR was mapped to the *CUX1* locus [31]. Cux1 deficiency negatively affects the expression of ATM/p53 pathway members [35]. *CUX1* deletions in sAML must have a weaker effect on DNA damage response pathways compared with mutated *TP53* as we did not observe influence of *CUX1* deletions on OS of patients, whereas mutated *TP53* negatively impacts OS. Our study convincingly showed for the first time that mutated *TP53* in sAML is a strong independent prognostic factor of poor survival. Previous reports showed worse survival of *TP53* mutated dnAML patients with complex karyotype [36,37]. The importance of *p53* in the leukemogenesis of sAML is further supported by our finding that gains of 1q32.1 harboring the *MDM4* (known inhibitor of p53) were found exclusively in secondary AML. They often co-occurred with del7q (*CUX1*) ($N = 4$ cases), suggesting that DNA damage response pathway defects play a crucial role in sAML pathogenesis.

A recent report of complex clonal architecture in sAML, showing various rare mutations in each patient [14] highlights the need for identification of robust genetic markers with relatively high frequency. Since monoallelic *TP53* mutations are detectable in chronic phase MPN [22] and MDS [38] (3.5% and 7.5% of the cases, respectively), screening for *TP53* mutations in chronic myeloid malignancies could be a useful marker predicting leukemic transformation.

The analysis of pair-wise association of genetic aberrations identified several new collaborating defects, such as del5q and del12p (*ETV6*), which require further functional validation. We confirmed previous reports that *TP53* mutations are often found together with 5q deletions [39]. It is unclear at this point to what extent the poor prognosis associated with mutated *TP53* is influenced by 5q deletions.

The high rate of karyotype complexity and its nonsignificant effect on OS in sAML suggests that many chromo-

TABLE II. Multivariate Analysis of Overall Survival Duration of Patients with Secondary Acute Myeloid Leukemia

Variables	Hazard ratio	95% CI	*P*
TP53 mutation	2.67	1.33–5.37	0.006
Complex karyotype	1.31	0.69–2.49	0.411
Del7q (*CUX1*)	0.76	0.33–1.75	0.527
Del7p (*IKZF1*)	1.27	0.43–3.78	0.659
Age	2.18	0.97–4.90	0.059

TABLE III. Clinical and Genetic Characteristics of De Novo Acute Myeloid Leukemia Patients with Complex Karyotype

UPN	Sex	Age	FAB	Overall survival (months)	Complex karyotype	*NPM1*	*FLT3*	*IDH1/2*	*RUNX1*	*NRAS*	*CBL*	*TP53*	del17p	del7q (*CUX1*)	del3p (*FOXP1*)	del7p (*IKZF1*)	del12p (*ETV6*)
4	F	30	M0	0.5	Yes	Wt	Wt	Wt	Wt	Wt	Wt	Wt	–	–	–	Yes	–
11	M	41	M5	3	Yes	Wt	Wt	Wt	Wt	Wt	Wt	Wt	–	–	–	–	–
42	F	30	M2	5	Yes	Wt	Wt	Wt	Wt	G13D	Wt	Wt	–	–	–	–	–
44	F	61	M2	3	Yes	Wt	Wt	Wt	Wt	Wt	Wt	Wt	Yes	–	Yes	Yes	Yes
51	F	63	M6	0.5	Yes	Wt	Wt	Wt	S418Pfs	Wt	Wt	Y220C	Yes	Yes	–	–	–
67	M	55	M2	3	Yes	Wt	Wt	Wt	Wt	Wt	Wt	Wt	Yes	–	–	–	–
68	F	66	M2	1	Yes	Wt	Wt	Wt	Wt	G12C	Wt	S215G/R337C	–	Yes	–	yes	Yes
72	F	82	M4	2	Yes	Wt	Wt	Wt	Wt	G12D	Wt	Wt	–	Yes	Yes	yes	Yes
86	M	50	M1	3.4	Yes	Wt	Wt	Wt	Wt	Wt	Wt	Wt	Yes	–	Yes	–	Yes
104	M	60	M4	10.6	Yes	Wt	Wt	R132C	Wt	Wt	Wt	G279E	Yes	–	Yes	–	–
121	M	70	M0	4.3	Yes	Wt	Wt	Wt	Wt	G12D	R420V	Wt	–	–	Yes	–	Yes

somal lesions represent functionally irrelevant aberrations and that chromosomal instability is a hallmark of sAML pathogenesis. The karyotype complexity in sAML is likely due to longer clonal evolution before leukemic transformation. A recent study showed that therapies administered during chronic phase of MPN do not associate with increased risk of AML transformation, and that 25% of therapy-naive patients develop AML [40]. However, a different study with longer observation time reported increased transformation rates in patients treated with pipobroman or hydroxyurea [41]. It is likely that certain patient-specific adaptations to therapies might utilize pathways promoting leukemic transformation, such as the DNA damage response pathway. In contrast to sAML, complex karyotype significantly contributed to poor OS in the dnAML patients as previously reported [42]. Interestingly, the majority of dnAML patients with complex karyotype exhibited genetic features typical for either chronic phase MPN or sAML. These lesions included mutated *TP53*, presence of *CUX1* deletions, and deletions of *FOXP1* and *ETV6* previously shown to be associated with chronic MPN [31]. This observation suggests that a number of patients diagnosed as dnAML with complex karyotype might in fact be sAML patients with a previously undiagnosed MPN or MDS either due to masked chronic phase phenotypes or absence of hematological examination preceding leukemic transformation.

We have shown that the genetic features of AML arising de novo substantially differ from post-MPN and post-MDS AML. Our data reinforce the fact that dnAML and sAML should be treated as separate AML subtypes. Furthermore, genetic resemblance of dnAML with complex karyotype and sAML indicate that these patients might constitute a relatively homogenous group for therapeutic intervention. This study demonstrates the use of genetic stratification of AML patients and suggests that despite the immense genetic heterogeneity among AML patients, certain markers have strong influence on patients' survival.

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